

WHAT IS CLAIMED IS:

1. A method for detecting a single nucleotide polymorphism in a target comprising, in an isothermal nucleic acid amplification reaction:

5        a) hybridizing a detector primer to the target, wherein the detector primer comprises a diagnostic nucleotide for the single nucleotide polymorphism about one to four nucleotides from a 3' terminal nucleotide of the detector primer which is complementary to the target sequence;

10        b) amplifying the target by hybridization and extension of the detector primer;

15        c) determining an efficiency of detector primer extension, and;

20        d) detecting the presence or absence of the single nucleotide polymorphism based on the efficiency of detector primer extension.

2. The method of Claim 1 wherein the ~~single~~ nucleotide polymorphism is identified using the detector primer.

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Sub A2        3. The method of Claim 2 wherein the single nucleotide polymorphism is identified ~~using~~ using two or more detector primers, each comprising a different diagnostic nucleotide.

20        4. The method of Claim 3 wherein two detector primers are used to identify which of two possible alleles is present in the target sequence.

25        5. The method of Claim 3 wherein four detector primers are used to identify the nucleotide present in the target sequence at the position of the single nucleotide polymorphism.

6. The method of Claim 3 wherein each of the multiple detector primers has a different 5' tail sequence.

7. The method of Claim 1 wherein the detector primer further comprises a nucleotide which forms a nondiagnostic mismatch with the target sequence.

8. The method of Claim 7 wherein the nondiagnostic nucleotide is positioned within fifteen nucleotides of the diagnostic nucleotide in the detector primer.

35        9. The method of Claim 8 wherein the nondiagnostic nucleotide is positioned 1-5 nucleotides from the diagnostic nucleotide in the detector primer.

10. The method of Claim 9 wherein the nondiagnostic nucleotide is adjacent to the diagnostic nucleotide in the detector primer.

11. The method of Claim 7 wherein the detector primer is about 15-36 nucleotides long.

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12. The method of Claim 11 wherein the detector primer is about 18-24 nucleotides long.

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13. The method of Claim 1 wherein the isothermal amplification reaction is selected from the group consisting of SDA, 3SR, NASBA and TMA.

14. The method of Claim 1 wherein the detector primer is about 12-50 nucleotides long.

15. The method of Claim 14 wherein the detector primer is about 12-24 nucleotides long.

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16. The method of Claim 15 wherein the detector primer is about 12-19 nucleotides long.

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17. The method of Claim 1 wherein the presence or absence of the single nucleotide polymorphism is detected by means of a label associated with the detector primer.

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18. The method of Claim 17 wherein the label becomes detectable upon extension of the detector primer or produces a change in signal upon extension of the detector primer.

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19. The method of Claim 18 wherein the label is a fluorescent donor/quencher dye pair and a decrease in donor dye fluorescence is detected as an indication of the presence of the single nucleotide polymorphism.

20. The method of Claim 19 wherein a change in fluorescence polarization is detected as an indication of the presence of the single nucleotide polymorphism.

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21. The method of Claim 1 wherein the efficiency of detector primer extension is determined quantitatively.

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22. The method of Claim 1 further comprising, prior to amplifying, displacing the hybridized detector primer from the target by extension of an upstream primer.